NOVEL COMPOUNDS

The present invention relates to antagonists of human interleukin 4 (IL4) and/or human interleukin 13 (IL13) for the treatment of conditions resulting from undesirable actions of IL4 and/or IL13 such as certain IgE mediated allergic diseases, T cell mediated autoimmune conditions and inappropriate immune responses to infectious agents.

Interleukins are secreted peptide mediators of the immune response. Each of the known interleukins has many effects on the development, activation, proliferation and differentiation of cells of the immune system. IL4 has a physiological role in such functions, but can also contribute to the pathogenesis of disease. In particular IL4 is associated with the pathway of B lymphocyte development that leads to the generation of IgE antibodies that are the hallmark of allergic diseases such as extrinsic asthma, rhinitis, allergic conjunctivitis, atopic dermatitis and anaphylaxis. IL4 can also act as a general growth and differentiation factor for T lymphocytes that may contribute to tissue damage in certain autoimmune conditions such as insulin dependent diabetes, multiple sclerosis and rheumatoid arthritis and in graft rejection. IL4 can also suppress the generation of cell-mediated responses required for the control of infectious disease. Antagonism of the effect of IL4 on T or B lymphocytes can therefore be expected to have beneficial effects on such diseases. IL13 has been recently identified and shares similarity in many of the biological properties of IL4 (Minty, A. *et al* (1993), Nature **362**, 248-250) including some aspect(s) of receptor structure/function (Aversa, G. *et al* (1993), J. Exp. Med. **178**, 2213-2218).

Human IL4 consists of a single polypeptide chain of 129 amino acids with 2 possible N-glycosylation sites and 6 cysteines involved in 3 disulphide bridges (Le, H.V. *et. al.*, (1988), J. Biol. Chem. **263**, 10817-10823). The amino acid sequence of IL4 and the positions of these disulphide bridges are known (Carr, C. *et al.*, (1991) Biochemistry **30**, 1515-1523).

```
                                        10
HIS-LYS-CYS-ASP-ILE-THR-LEU-GLN-GLU-ILE-ILE-LYS-THR-LEU-ASN-

                    20                                      30
SER-LEU-THR-GLU-GLN-LYS-THR-LEU-CYS-THR-GLU-LEU-THR-VAL-THR-

                                        40
ASP-ILE-PHE-ALA-ALA-SER-LYS-ASN-THR-THR-GLU-LYS-GLU-THR-PHE-

                    50                                      60
CYS-ARG-ALA-ALA-THR-VAL-LEU-ARG-GLN-PHE-TYR-SER-HIS-HIS-GLU-

                                    70
LYS-ASP-THR-ARG-CYS-LEU-GLY-ALA-THR-ALA-GLN-GLN-PHE-HIS-ARG-
```

```
                         80                                      90
HIS-LYS-GLN-LEU-ILE-ARG-PHE-LEU-LYS-ARG-LEU-ASP-ARG-ASN-LEU-

                                         100
TRP-GLY-LEU-ALA-GLY-LEU-ASN-SER-CYS-PRO-VAL-LYS-GLU-ALA-ASN-

                        110                                      120
GLN-SER-THR-LEU-GLU-ASN-PHE-LEU-GLU-ARG-LEU-LYS-THR-ILE-MET-

                                     129
ARG-GLU-LYS-TYR-SER-LYS-CYS-SER-SER
```

The disulphide bridges are between residues 3 and 127, 24 and 65, and 46 and 99. The molecular weight of IL4 varies with the extent of glycosylation from 15KDa (no glycosylation) to 60KDa or more (hyperglycosylated IL4).

The DNA sequence for human IL4 has also been described by Yokota, T. *et. al.*, P.N.A.S. 1986 **83** 5894-5898.

WO 93/10235 describes certain mutants of IL4 which are IL4 antagonists or partial antagonists.

EP-A-0 464 533 discloses fusion proteins comprising various portions of the constant region of immunoglobulin molecules together with another human protein or part thereof.

The present invention provides a soluble protein having IL4 and/or IL13 antagonist or partial antagonist activity, comprising an IL4 mutant or variant fused to least one human immunoglobulin constant domain or fragment thereof.

The term "mutant or variant" encompasses any molecule such as a truncated or other derivative of the IL4 protein which retains the ability to antagonise IL4 and/or IL13 following internal administration to a human. Such other derivatives can be prepared by the addition, deletion, substitution, or rearrangement of amino acids or by chemical modifications thereof.

DNA polymers which encode mutants or variants of IL4 may be prepared by site-directed mutagenesis of the cDNA which codes for IL4 by conventional methods such as those described by G. Winter *et al* in Nature 1982, **299**, 756-758 or by Zoller and Smith 1982; Nucl. Acids Res., **10**, 6487-6500, or deletion mutagenesis such as described by Chan and Smith in Nucl. Acids Res., 1984, **12**, 2407-2419 or by G. Winter *et al* in Biochem. Soc. Trans., 1984; **12**, 224-225 or polymerase chain reaction such as described by Mikaelian and Sergeant in Nucleic Acids Research, 1992, **20**, 376.

As used herein. "having IL4 and/or IL13 antagonist or partial antagonist activity" means that, in the assay described by Spits *et al* (J. Immunology **139**, 1142 (1987)), IL4-stimulated T cell proliferation is inhibited in a dose-dependent manner.

Suitable IL4 mutants are disclosed in WO 93/10235, wherein at least one amino acid, naturally occuring in wild type IL4 at any one of positions 120 to 128 inclusive, is replaced by a different natural amino acid. In particular, the tyrosine naturally occurring at position 124 may be replaced by a different natural amino acid, such as glycine or, more preferably, aspartic acid.

The immunoglobulin may be of any subclass (IgG, IgM, IgA, IgE), but is preferably IgG, such as IgG1, IgG3 or IgG4. The said constant domain(s) or fragment thereof may be derived from the heavy or light chain or both. The invention encompasses mutations in the immunoglobulin component which eliminate undesirable properties of the native immunoglobulin, such as Fc receptor binding and/or introduce desirable properties such as stability. For example, Angal S., King D.J., Bodmer M.W., Turner A., Lawson A.D.G., Roberts G., Pedley B. and Adair R., Molecular Immunology vol30pp105-108, 1993, describe an IgG4 molecule where residue 241 (Kabat numbering) is altered from serine to proline. This change increases the serum half-life of the IgG4 molecule. Canfield S.M. and Morrison S.L., Journal of Experimental Medicine vol173pp1483-1491, describe the alteration of residue 248 (Kabat numbering) from leucine to glutamate in IgG3 and from glutamate to leucine in mouse IgG2b. Substitution of leucine for glutamate in the former decreases the affinity of the immunoglobulin molecule concerned for the FcγRI receptor, and substitution of glutamate for leucine in the latter increases the affinity. EP0307434 discloses various mutations including an L to E mutation at residue 248 (Kabat numbering) in IgG.

The constant domain(s) or fragment thereof is preferably the whole or a substantial part of the constant region of the heavy chain of human IgG, most preferably IgG4. In one aspect the IgG component consists of the CH2 and CH3 domains and the hinge region of IgG1 including cysteine residues contributing to inter-heavy chain disulphide bonding, for example residues 11 and 14 of the IgG1 hinge region (Frangione B. and Milstein C., Nature vol216pp939-941, 1967). Preferably the IgG1 component consists of amino acids corresponding to residues 1-4 and 6-15 of the hinge, 1-110 of CH2 and 1-107 of CH3 of IgG1 described by Ellison J., Berson B. and Hood L. E., Nucleic Acids Research vol10, pp4071-4079, 1982. Residue 5 of the hinge is changed from cysteine in the published IgG1 sequence to alanine by alteration of TGT to GCC in the nucleotide sequence. In another aspect the IgG component is derived from IgG4, comprising the CH2 and CH3 domains and the hinge region including cysteine residues contributing to inter-heavy chain disulphide bonding, for example residues 8 and 11 of the IgG4 hinge region (Pinck J.R. and Milstein C., Nature vol216pp941-942, 1967). Preferably the IgG4 component consists of amino acids corresponding to residues 1-12 of the

hinge, 1-110 of CH2 and 1-107 of CH3 of IgG4 described by Ellison J., Buxbaum J. and Hood L., DNA vol1pp11-18, 1981. In one example of a suitable mutation in IgG4, residue 10 of the hinge (residue 241, Kabat numbering) is altered from serine (S) in the wild type to proline (P) and residue 5 of CH2 (residue 248, Kabat numbering) is altered from leucine (L) in the wild type to glutamate (E).

Fusion of the IL4 mutant or variant to the Ig constant domain or fragment is by C-terminus of one component to N-terminus of the other. Preferably the IL4 mutant or variant is fused via its C-terminus to the N-terminus of the Ig constant domain or fragment.

In a preferred aspect, the amino acid sequence of the fusion protein of the invention is represented by SEQ ID No:4, SEQ ID No:7 or SEQ ID No:10.

In a further aspect, the invention provides a process for preparing a compound according to the invention which process comprises expressing DNA encoding said compound in a recombinant host cell and recovering the product.

The DNA polymer comprising a nucleotide sequence that encodes the compound also forms part of the invention.

In a preferred aspect the DNA polymer comprises or consists of the sequence of SEQ ID No:3, SEQ ID No:6 or SEQ ID No:9.

The process of the invention may be performed by conventional recombinant techniques such as described in Maniatis *et. al.*, Molecular Cloning - A Laboratory Manual; Cold Spring Harbor, 1982 and DNA Cloning vols I, II and III (D.M. Glover ed., IRL Press Ltd).

In particular, the process may comprise the steps of:

i) preparing a replicable expression vector capable, in a host cell, of expressing a DNA polymer comprising a nucleotide sequence that encodes said compound;

ii) transforming a host cell with said vector;

iii) culturing said transformed host cell under conditions permitting expression of said DNA polymer to produce said compound; and

iv) recovering said compound.

The invention also provides a process for preparing the DNA polymer by the condensation of appropriate mono-, di- or oligomeric nucleotide units.

The preparation may be carried out chemically, enzymatically, or by a combination of the two methods, *in vitro* or *in vivo* as appropriate. Thus, the DNA polymer may be prepared by the enzymatic ligation of appropriate DNA fragments, by conventional methods such as those described by D. M. Roberts *et al* in Biochemistry 1985, **24**, 5090-5098.

The DNA fragments may be obtained by digestion of DNA containing the required sequences of nucleotides with appropriate restriction enzymes, by chemical synthesis, by enzymatic polymerisation on DNA or RNA templates, or by a combination of these methods.

Digestion with restriction enzymes may be performed in an appropriate buffer at a temperature of 20^{o}-$70^{o}C$, generally in a volume of 50μl or less with 0.1-10μg DNA.

Enzymatic polymerisation of DNA may be carried out *in vitro* using a DNA polymerase such as DNA polymerase I (Klenow fragment) in an appropriate buffer containing the nucleoside triphosphates dATP, dCTP, dGTP and dTTP as required at a temperature of 10^{o}-$37^{o}C$, generally in a volume of 50μl or less.

Enzymatic ligation of DNA fragments may be carried out using a DNA ligase such as T4 DNA ligase in an appropriate buffer at a temperature of $4^{o}C$ to ambient, generally in a volume of 50μl or less.

The chemical synthesis of the DNA polymer or fragments may be carried out by conventional phosphotriester, phosphite or phosphoramidite chemistry, using solid phase techniques such as those described in 'Chemical and Enzymatic Synthesis of Gene Fragments - A Laboratory Manual' (ed. H.G. Gassen and A. Lang), Verlag Chemie, Weinheim (1982),or in other scientific publications, for example M.J. Gait, H.W.D. Matthes, M. Singh, B.S. Sproat, and R.C. Titmas, Nucleic Acids Research, 1982, **10**, 6243; B.S. Sproat and W. Bannwarth, Tetrahedron Letters, 1983, **24**, 5771; M.D. Matteucci and M.H Caruthers, Tetrahedron Letters, 1980, **21**, 719; M.D. Matteucci and M.H. Caruthers, Journal of the American Chemical Society, 1981, **103**, 3185; S.P. Adams *et al.*, Journal of the American Chemical Society,1983, **105**, 661; N.D. Sinha, J. Biernat, J. McMannus, and H. Koester, Nucleic Acids Research, 1984, **12**, 4539; and H.W.D. Matthes *et al.*, EMBO Journal, 1984, **3**, 801. Preferably an automated DNA synthesizer is employed.

The DNA polymer is preferably prepared by ligating two or more DNA molecules which together comprise a DNA sequence encoding the compound. A particular process in accordance with the invention comprises ligating a first DNA molecule encoding a said IL4 mutant or variant and a second DNA molecule encoding a said immunoglobulin domain or fragment thereof.

The DNA molecules may be obtained by the digestion with suitable restriction enzymes of vectors carrying the required coding sequences or by use of polymerase chain reaction technology.

The precise structure of the DNA molecules and the way in which they are obtained depends upon the structure of the desired product. The design of a

suitable strategy for the construction of the DNA molecule coding for the compound is a routine matter for the skilled worker in the art.

The expression of the DNA polymer encoding the compound in a recombinant host cell may be carried out by means of a replicable expression vector capable, in the host cell, of expressing the DNA polymer. The expression vector is novel and also forms part of the invention.

The replicable expression vector may be prepared in accordance with the invention, by cleaving a vector compatible with the host cell to provide a linear DNA segment having an intact replicon, and combining said linear segment with one or more DNA molecules which, together with said linear segment, encode the compound, under ligating conditions.

The ligation of the linear segment and more than one DNA molecule may be carried out simultaneously or sequentially as desired.

Thus, the DNA polymer may be preformed or formed during the construction of the vector, as desired.

The choice of vector will be determined in part by the host cell, which may be prokaryotic, such as *E. coli*, or eukaryotic, such as mouse C127, mouse myeloma, chinese hamster ovary or Hela cells, fungi e.g. filamentous fungi or unicellular yeast or an insect cell such as Drosophila. The host cell may also be a transgenic animal. Suitable vectors include plasmids, bacteriophages, cosmids and recombinant viruses derived from, for example, baculoviruses, vaccinia or Semliki Forest virus.

The preparation of the replicable expression vector may be carried out conventionally with appropriate enzymes for restriction, polymerisation and ligation of the DNA, by procedures described in, for example, Maniatis et al., cited above. Polymerisation and ligation may be performed as described above for the preparation of the DNA polymer. Digestion with restriction enzymes may be performed in an appropriate buffer at a temperature of 20^{o}-$70^{o}C$, generally in a volume of $50\mu l$ or less with 0.1-$10\mu g$ DNA.

The recombinant host cell is prepared, in accordance with the invention, by transforming a host cell with a replicable expression vector of the invention under transforming conditions. Suitable transforming conditions are conventional and are described in, for example, Maniatis *et al.*, cited above, or "DNA Cloning" Vol. II, D.M. Glover ed., IRL Press Ltd, 1985.

The choice of transforming conditions is determined by the host cell. Thus, a bacterial host such as *E. coli* may be treated with a solution of $CaCl_2$ (Cohen *et al*, Proc. Nat. Acad. Sci., 1973, **69**, 2110) or with a solution comprising a mixture of RbCl, $MnCl_2$, potassium acetate and glycerol, and then with 3-[N-morpholino]-

propane-sulphonic acid, RbCl and glycerol. Mammalian cells in culture may be transformed by calcium co-precipitation of the vector DNA onto the cells.

The invention also extends to a host cell transformed with a replicable expression vector of the invention.

Culturing the transformed host cell under conditions permitting expression of the DNA polymer is carried out conventionally, as described in, for example, Maniatis *et al* and "DNA Cloning" cited above. Thus, preferably the cell is supplied with nutrient and cultured at a temperature below 45^{o}C.

The expression product is recovered by conventional methods according to the host cell. Thus, where the host cell is bacterial, such as *E. coli* it may be lysed physically, chemically or enzymatically and the protein product isolated from the resulting lysate. If the product is to be secreted from the bacterial cell it may be recovered from the periplasmic space or the nutrient medium. Where the host cell is mammalian, the product may generally be isolated from the nutrient medium.

The DNA polymer may be assembled into vectors designed for isolation of stable transformed mammalian cell lines expressing the product; e.g. bovine papillomavirus vectors or amplified vectors in chinese hamster ovary cells (DNA cloning Vol.II D.M. Glover ed. IRL Press 1985; Kaufman, R.J. et al., Molecular and Cellular Biology 5, 1750-1759, 1985; Pavlakis G.N. and Hamer, D.H., Proceedings of the National Academy of Sciences (USA) 80, 397-401, 1983; Goeddel, D.V. *et al.*, European Patent Application No. 0093619, 1983).

Compounds of the present invention have IL4 and/or IL13 antagonist activity and are therefore of potential use in the treatment of conditions resulting from undesirable actions of IL4 and/or IL13 such as IgE mediated allergic diseases and T cell mediated autoimmune conditions or chronic microbial infection.

The invention therefore further provides a pharmaceutical composition comprising a compound of the invention and a pharmaceutically acceptable carrier.

In use the compound will normally be employed in the form of a pharmaceutical composition in association with a human pharmaceutical carrier, diluent and/or excipient, although the exact form of the composition will depend on the mode of administration. The compound may, for example. be employed in the form of aerosol or nebulisable solution for inhalation or sterile solutions for parenteral administration.

The dosage ranges for administration of the compounds of the present invention are those to produce the desired effect on the IL4 and/or IL13 mediated condition, for example whereby IgE antibody mediated symptoms are reduced or progression of the autoimmune disease is halted or reversed. The dosage will generally vary with age, extent or severity of the medical condition and

contraindications, if any. The unit dosage can vary from less than 1mg to 300mg, but typically will be in the region of 1 to 20mg per dose, in one or more doses, such as one to six doses per day, such that the daily dosage is in the range 0.02-40mg/kg.

Compositions suitable for injection may be in the form of solutions, suspensions or emulsions, or dry powders which are dissolved or suspended in a suitable vehicle prior to use.

Fluid unit dosage forms are prepared utilising the compound and a pyrogen-free sterile vehicle. The compound, depending on the vehicle and concentration used, can be either dissolved or suspended in the vehicle. Solutions may be used for all forms of parenteral administration, and are particularly used for intravenous infection. In preparing solutions the compound can be dissolved in the vehicle, the solution being made isotonic if necessary by addition of sodium chloride and sterilised by filtration through a sterile filter using aseptic techniques before filling into suitable sterile vials or ampoules and sealing. Alternatively, if solution stability is adequate, the solution in its sealed containers may be sterilised by autoclaving. Advantageously additives such as buffering, solubilising, stabilising, preservative or bactericidal, suspending or emulsifying agents and/or local anaesthetic agents may be dissolved in the vehicle.

Dry powders which are dissolved or suspended in a suitable vehicle prior to use may be prepared by filling pre-sterilised drug substance and other ingredients into a sterile container using aseptic technique in a sterile area. Alternatively the drug and other ingredients may be dissolved in an aqueous vehicle, the solution is sterilised by filtration and distributed into suitable containers using aseptic technique in a sterile area. The product is then freeze dried and the containers are sealed aseptically.

Parenteral suspensions, suitable for intramuscular, subcutaneous or intradermal injection, are prepared in substantially the same manner, except that the sterile compound is suspended in the sterile vehicle, instead of being dissolved and sterilisation cannot be accomplished by filtration. The compound may be isolated in a sterile state or alternatively it may be sterilised after isolation, e.g. by gamma irradiation. Advantageously, a suspending agent for example polyvinylpyrrolidone is included in the composition to facilitate uniform distribution of the compound.

Compositions suitable for administration via the respiratory tract include aerosols, nebulisable solutions or microfine powders for insufflation. In the latter case, particle size of less than 50 microns, especially less than 10 microns, is preferred. Such compositions may be made up in a conventional manner and employed in conjunction with conventional administration devices.

In a further aspect there is provided a method of treating conditions resulting from undesirable actions of IL4 and/or IL13 which comprises administering to the sufferer an effective amount of a compound of the invention.

The invention further provides a compound of the invention for use as an active therapeutic substance, in particular for use in treating conditions resulting from undesirable actions of IL4 and/or IL13.

The invention also provides the use of a compound of the invention in the manufacture of a medicament for treating conditions resulting from undesirable actions of IL4 and/or IL13.

No unexpected toxicological effects are expected when compounds of the invention are administered in accordance with the present invention.

The following Examples illustrate the invention.

Example 1 **IL4.Y124D/IgG1 fusion protein**

The construction of an IL4.Y124D/IgG1 chimeric cDNA, the expression of the corresponding protein in a mammalian expression system and its activity are described.

1. Construction of DNA coding for fusion protein

(a) Construction of IL4.Y124D coding region

A variant of the human IL4 gene, which has been described (Kruse, N, Tony, H-P and Sebald, W. EMBO Journal <u>11</u>: 3237 [1992]) in which residue 124 in the protein has been mutated from tyrosine in the wild type to aspartic acid, was produced by PCR mutagenesis of the human IL4 cDNA (purchased from British Biotechnology). The IL4.Y124D cDNA was inserted into the expression vector pTR312, using the HindIII and BglII sites, (M J Browne, J E Carey, C G Chapman, A W R Tyrrell, C Entwisle, G M P Lawrence, B Reavy, I Dodd, A Esmail & J H Robinson. Journal of Biological Chemistry <u>263</u>: 1599, [1988]) to form the plasmid pDB906.

To amplify the IL4.Y124D molecule and add convenient restriction sites at each end for subcloning, a PCR reaction was performed using 20ng of the pDB906 plasmid as the substrate. PCR primers were designed to include restriction enzyme sites, flanked by 10-15 nucleotide base pairs to "anchor" the primers at each end. The primer sequences were as follows:

1) 5' CGA ACC ACT GAA TTC CGC ATT GCA GAG ATA 3'
(includes an EcoRI restriction site, GAATTC)

2) 5' CAC AAA GAT CCT TAG GTA CCG CTC GAA CAC TTT GA 3'
(includes a KpnI restriction site, GGTACC)

Primers were used at a final concentration of 5ng/μl, and dNTPs were added at a final concentration of 0.2mM in a total reaction volume of 100μl. 31 cycles of PCR were performed. Cycles consisted of a denaturation step of 1 minute at 94°C, an annealing step of 1 minute 30 seconds at 50°C, and an elongation step of 1 minute 30 seconds at 72°C. On cycle 1 denaturation was extended to 5 minutes and on the final cycle elongation was extended to 7 minutes. 2.5 units of the Taq polymerase enzyme from Advanced Biotechnologies were used in the PCR reaction. A PCR product of 587bp was produced. This was purified using the Promega "Magic PCR cleanup" kit, and then digested with EcoRI and KpnI in react buffer 4 (all restriction enzymes were obtained from GibcoBRL.), to generate 'sticky ends'. After 4 hours 30 minutes at 37°C, the reaction was heated to 70°C for 10 minutes and then ethanol precipitated. Analysis of the resulting DNA by agarose gel electrophoresis showed the presence of three bands of approximately 570bp, 463bp and 100bp . The 570bp fragment represents the full-length IL4.Y124D variant of IL4 and was present because the digest was incomplete. The two smaller fragments were produced due to the presence of an EcoRI site within the IL4.Y124D cDNA. The 570bp band was purified by the Geneclean ™ procedure, and ligated into Bluescript KS+ ™ which was prepared by digestion with EcoRI and KpnI followed by Geneclean ™. A Bluescript KS+/IL4.Y124D recombinant was thus generated. Large amounts of this recombinant DNA were produced using the Promega "Magic Maxiprep" method. The IL4.Y124D insert was excised from the Bluescript recombinant using SmaI and KpnI. 20μg recombinant DNA was incubated with 25 units SmaI in react buffer 4, at 30°C overnight. 25 units of KpnI were then added to the digest, which was incubated at 37°C for 5 hours. The resulting fragment of approximately 580bp was purified by Geneclean ™ to generate an IL4.Y124D/SmaI/KpnI fragment.

(b) Construction of IgG1 coding region

The COSFcLink vector (Table 1) contains human IgG1 cDNA encoding amino acids 1-4 and 6-15 of the hinge, 1-110 of CH2 and 1-108 of CH3 described by Ellison J., Berson B. and Hood L. E., Nucleic Acids Research vol10, pp4071-4079, 1982. Residue 5 of the hinge is changed from cysteine in the published IgG1 sequence to alanine by alteration of TGT to GCC in the nucleotide sequence. This was cloned from the human IgG plasma cell leukemia ARH-77 (American Type

Tissue Collection), using RT-PCR and fully sequenced to confirm identity with the published sequence [patent application publication WO 92/00985]

The construction of COSFc began with a pUC18 vector containing the human IgG1 cDNA above (pUC18-Fc), which was digested with KpnI and SacII, deleting the CH1, hinge and part of CH2. The deleted region was replaced with a PCR amplified fragment containing the hinge-CH2 region as follows. Using the following PCR primers:

5' TCG AGC TCG GTA CCG AGC CCA AAT CGG CCG ACA AAA CTC ACA C 3'

and

5' GTA CTG CTC CTC CCG CGG CTT TGT CTT G 3'

A DNA fragment containing the hinge-CH2 region was amplified from pUC18-Fc, digested with KpnI and SacII, gel purified and cloned into the KpnI/SacII digested pUC18-Fc vector. The Cys, which occurs at position 230 (Kabat numbering; Kabat et al., "Sequences of Proteins of Immunological Interest, 5th Edition, US Department of Health and Human Services, NIH Publication No. 91-3242 (1991)) of the IgG1 heavy chain, was altered to an Ala through a TGT to GCC substitution in the nucleotide sequence. An altered DNA sequence in one of the PCR primers introduced a unique KpnI site at the 5' end of the hinge. The resulting plasmid was called pUC18Fcmod, and the junctions and PCR amplified region were sequenced for confirmation.

The entire hinge-CH2-CH3 insert in pUC18-Fcmod was removed in a single DNA fragment with KpnI and XbaI, gel purified, and ligated into SFcR1Cos4 cut with KpnI and XbaI to create COSFc.

SFcR1Cos4 is a derivative of pST4DHFR (Deen, K , McDougal. JS, Inacker, R. Folena-Wasserman, G, Arthos, J, Rosenberg, J, Maddon, PJ, Axel, R, and Sweet, RW. Nature <u>331</u>: 82 [1988]) and contains the soluble Fc receptor type I (sFcR1) inserted between the cytomegalovirus (CMV) promoter and bovine growth hormone (BGH) polyadenylation regions, and also contains the dihydrofolate reductase (DHFR) cDNA inserted between the β-globin promoter and SV40 polyadenylation regions, an SV40 origin of replication, and an ampicillin resistance gene for growth in bacteria. Cutting the vector with KpnI and XbaI removes the sFcR1 coding region, so that the COSFc vector contains the hinge-CH2-CH3 region inserted between the CMV promoter and BGH polyA regions.

The COSFcLink vector was made from COSFc by inserting an oligonucleotide linker at the unique EcoRI site of the vector, which recreates this

EcoRI site, and also introduces BstEII, PstI and EcoRV cloning sites. The oligonucleotides used were:

5' AATTCGGTTACCTGCAGATATCAAGCT 3'
3' GCCAATGGACGTCTATAGTTCGATTAA 5'

The junction was sequenced to confirm orientation in the vector. The size of the final vector is 6.37 kb.

(c) Construction of DNA coding for fusion protein.

To insert the IL4.Y124D cDNA, the COSFcLink vector was prepared by digesting with EcoRV and KpnI as follows: 5μg DNA was incubated with 15 units EcoRV in react 2 at 37°C for 5 hours, followed by ethanol precipitation. The resulting DNA was digested with KpnI in react 4 at 37°C for 3 hours, and ethanol precipitated. The IL4.Y124D/SmaI/KpnI and the COSFcLink/EcoRV/KpnI fragments were ligated together to form plasmid pDB951, which encodes the IL4.Y124D/IgG1 fusion protein. The ligation was achieved using an Amersham DNA ligation kit, product code RPN 1507, the reactions being incubated at 16°C overnight. The ligation reaction products were transformed into Promega JM109 competent cells (high efficiency) and plated onto Luria Broth agar containing ampicillin at 50μg/ml. Transformants were cultured in Luria Broth (containing ampicillin at 50μg/ml) and DNA prepared using Promega "Magic Minipreps". Production of an IL4.Y124D/COSFcLink recombinant DNA was verified by restriction digests and DNA sequencing. The complete IL4.Y124D sequence and the junctions with the COSFcLink DNA were confirmed by DNA sequencing (Table 2). The coding sequence of the recombinant IL4.Y124D/IgG1 DNA is shown in Table 3 and the amino acid sequence of the fusion protein is shown in Table 4. The IL4.Y124D/COSFcLink recombinant DNA was prepared and purified using caesium chloride gradients and the DNA used to transiently transfect HeLa cells.

2. Expression of the fusion protein

HeLa cells were grown in MEMα medium (Gibco) with 10% foetal calf serum and 1% glutamine. For the assay, 1 x 10^6 HeLa cells were seeded in 15mls RPMI-1640 medium with 10% newborn calf serum, 1% glutamine ("seeding medium"), in a 75cm^2 flask, four days prior to transfection. On the day prior to transfection, a further 12.5mls seeding medium was added to each flask. On the day of transfection, the medium was changed to 15mls of "transfection medium"

(MEM medium with Earle's salts containing 10% newborn calf serum and 1% non essential amino acids), at time zero. At time +3 hours, 25μg of the appropriate DNA in 0.125M $CaCl_2$, 1x HBS (HEPES buffered saline) was added to the cells. At time +7 hours, the cells were subjected to a glycerol shock (15%v/v) and then left to incubate overnight in 12.5mls seeding medium containing 5mM sodium butyrate. The next day the cells were washed with PBS (Dulbecco's phosphate buffered saline) and 12.5mls "harvest medium" (RPMI-1640 with 2% of a 7.5% stock sodium bicarbonate solution) was added. After a further 24 hour incubation, the supernatants were removed, centrifuged at 1000rpm for 5 minutes to remove cell debris and stored at either 4°C or -20°C.

3. Biological Activity

For assay of supernatant for IL4 antagonist activity: using the method described in Spits et al., J. Immunology 139, 1142 (1987), human peripheral blood lymphocytes were incubated for three days with phytohaemagluttinin, a T cell mitogen, to upregulate the IL4 receptor. The resultant blast cells were then stimulated for a further three days with IL4. Proliferation was measured by the incorporation of 3H thymidine.

The IL4.Y124D/IgG1 chimera inhibited ^{3}H thymidine incorporation by human peripheral blood T lymphocytes stimulated with 133pM IL4 in a dose dependent manner.

Example 2 IL4.Y124D/IgG4 fusion protein

1. Construction of DNA coding for fusion protein

PCR was performed to amplify the IL4.Y124D coding region and introduce a silent nucleotide substitution at the 3' end which creates a XhoI site. As substrate for the PCR reaction 20ng of linearised pDB951 plasmid (Example 1.1(c)) was used. The oligonucleotide primers used were as follows:

1) 5' CAC AAG TGC GAT ATC ACC TTA CAG GAG ATC 3'
(includes an EcoRV restriction site, GATATC)

2) 5' CTC GGT ACC GCT CGA GCA CTT TGA GTC TTT 3'
(includes a XhoI restriction site, CTCGAG).

A second PCR reaction was performed to amplify the hinge-CH2-CH3 fragment of the human IgG4 heavy chain. The substrate for this was a synthetic human IgG4 heavy chain cDNA, the sequence of which is described in Table 5, and is based on the Genbank sequence GB:HUMIGCD2 (Ellison J., Buxbaum J. and Hood L.E., DNA 1:11-18, 1981). Numerous silent substitutions were made to the published nucleotide sequence. The gene was assembled by combining two 0.5Kb synthetic DNA fragments. Each 0.5Kb fragment was made by annealing a series of overlapping oligonucleotides and then filling in the gaps by PCR. The two 0.5Kb fragments were joined at the SacII site and inserted into the pCR2 vector. A 1.0Kb ApaI-BglII fragment containing the entire constant region was isolated and ligated into an expression vector, pCD, containing a humanized IL4 specific variable region. This construct was used as the PCR substrate to amplify the hinge-CH2-CH3 region of IgG4.

The oligonucleotide primers used for amplification of the IgG4 hinge-CH2-CH3 region were as follows:

1) 5' GGT GGA CAA CTC GAG CGA GTC CAA ATA TGG 3'
(includes a XhoI restriction site, CTCGAG)

2) 5' TTA CGT AGA TCT AGA CTA CAC TCA TTT ACC 3'
(includes an XbaI site, TCTAGA).

The conditions for both PCR reactions were as described for the derivation of pDB951. Briefly, primers were used at 5ng/μl, and dNTPs at a final concentration of 0.2mM in a total reaction volume of 100μl. 2.5 Units of Taq polymerase enzyme from Advanced Biotechnologies were used and 31 cycles of PCR performed. Cycles consisted of a denaturation step of 1 minute at 94°C, an annealing step of 1 minute 30 seconds at 50°C, and an elongation step of 1 minute 30 seconds at 72°C. On cycle 1 denaturation was extended to 5 minutes and on the final cycle elongation was extended to 7 minutes.

PCR products of approximately 700bp (hinge-CH2-CH3 of IgG4) and 400bp (IL4.Y124D) were obtained and purified using the Promega "Magic PCR cleanup" kit. The purified PCR reactions were then digested with the following enzymes to create "sticky ends": XhoI and XbaI for IgG4 and EcoRV and XhoI for IL4.Y124D. The digests were incubated at 37°C for 3 hours and then ethanol precipitated. The resulting DNAs were analysed by gel electrophoresis and gave sizes of approximately 690bp (hinge-CH2-CH3 of IgG4) and 370bp (IL4.Y124D).

A vector was prepared into which to ligate the hinge-CH2-CH3 of IgG4 and IL4.Y124D PCR fragments by digesting pDB951 (IL4.Y124D in COSFcLink) with EcoRV and XbaI to remove most of the IL4.Y124D/IgG1 fusion molecule. The only part remaining is approximately 75bp at the 5' end of IL4, which is not present in the IL4.Y124D EcoRV/XhoI fragment produced by PCR amplification. 5μg of pDB951 DNA was digested in a total volume of 30μl using react 2 buffer (GibcoBRL). The resulting 5.8Kb DNA fragment was purified using the Geneclean TM procedure.

The three fragments described (IL4.Y124D EcoRV/XhoI, hinge-CH2-CH3 of IgG4 XhoI/XbaI and the 5.8Kb fragment resulting from EcoRV/XbaI digestion of pDB951) were ligated together to form plasmid pDB952, which encodes the IL4.Y124D/IgG4 fusion protein. The ligation was carried out using a DNA ligation kit from Amersham (product code RPN 1507), incubating the reactions at 16°C overnight. The ligation reaction products were transformed into Promega JM109 competent cells (high efficiency) and plated onto Luria Broth agar containing ampicillin at 50μg/ml. Transformants were cultured in Luria Broth (containing ampicillin at 50μg/ml) and DNA prepared using Promega "Magic Minipreps". Production of an IL4.Y124D/IgG4 recombinant DNA was verified by restriction digests, and the complete IL4.Y124D and hinge-CH2-CH3 IgG4 regions were verified by DNA sequencing. Table 6 describes the sequence of the coding region only of the IL4.Y124D/IgG4 fusion molecule, and Table 7 contains the amino acid sequence of the fusion protein. The IL4.Y124D/IgG4 recombinant DNA was prepared and purified using caesium chloride gradients and the DNA used to transiently transfect HeLa cells.

2. Expression of the fusion protein

HeLa cells were grown in MEMα medium (Gibco) with 10% foetal calf serum and 1% glutamine. For the assay, 1 x 10^6 HeLa cells were seeded in 15mls RPMI-1640 medium with 10% newborn calf serum, 1% glutamine ("seeding medium"), in a 75cm^2 flask, four days prior to transfection. On the day prior to transfection, a further 12.5mls seeding medium was added to each flask. On the day of transfection, the medium was changed to 15mls of "transfection medium" (MEM medium with Earle's salts containing 10% newborn calf serum and 1% non essential amino acids), at time zero. At time +3 hours, 25μg of the appropriate DNA in 0.125M $CaCl_2$, 1x HBS (HEPES buffered saline) was added to the cells. At time +7 hours, the cells were subjected to a glycerol shock (15%v/v) and then left to incubate overnight in 12.5mls seeding medium containing 5mM sodium butyrate. The next day the cells were washed with PBS (Dulbecco's phosphate

buffered saline) and 12.5mls "harvest medium" (RPMI-1640 with 2% of a 7.5% stock sodium bicarbonate solution) was added. After a further 24 hour incubation, the supernatants were removed, centrifuged at 1000rpm for 5 minutes to remove cell debris and stored at either 4°C or -20°C.

3. Biological Activity

For assay of supernatant for IL4 antagonist activity: using the method described in Spits et al., J. Immunology 139, 1142 (1987), human peripheral blood lymphocytes were incubated for three days with phytohaemagluttinin, a T cell mitogen, to upregulate the IL4 receptor. The resultant blast cells were then stimulated for a further three days with IL4. Proliferation was measured by the incorporation of 3H thymidine.

The IL4.Y124D/IgG4 chimera inhibited ^{3}H thymidine incorporation by human peripheral blood T lymphocytes stimulated with 133pM IL4 in a dose dependent manner.

Example 3 IL4.Y124D/IgG4 PE fusion protein

1. Construction of DNA coding for fusion protein

PCR is performed to amplify the IL4.Y124D coding region and introduce a silent nucleotide substitution at the 3' end which creates a XhoI site as described in Example 2.

A second PCR reaction is performed to amplify the hinge-CH2-CH3 fragment of the human IgG4 heavy chain PE variant. In IgG4 PE, residue 10 of the hinge (residue 241, Kabat numbering) is altered from serine (S) in the wild type to proline (P) and residue 5 of CH2 (residue 248, Kabat numbering) is altered from leucine (L) in the wild type to glutamate (E). Angal S., King D.J., Bodmer M.W., Turner A., Lawson A.D.G., Roberts G., Pedley B. and Adair R., Molecular Immunology vol30pp105-108, 1993, describe an IgG4 molecule where residue 241 (Kabat numbering) is altered from serine to proline. This change increases the serum half-life of the IgG4 molecule.

The IgG4 PE variant was created using PCR mutagenesis on the synthetic human IgG4 heavy chain cDNA described in Table 5, and was then ligated into the pCD expression vector. It was this plasmid which was used as the substrate for the PCR reaction amplifying the hinge-CH2-CH3 fragment of IgG4 PE. The sequence of the IgG4 PE variant is described in Table 8. The residues of

the IgG4 nucleotide sequence which were altered to make the PE variant are as follows:

referring to Table 8:

residue 322 has been altered to "C" in the PE variant from "T" in the wild type;

residue 333 has been altered to "G" in the PE variant from "A" in the wild type; and

residues 343-344 have been altered to "GA" in the PE variant from "CT" in the wild type.

Oligonucleotide primers are used for amplification of the IgG4 PE variant hinge-CH2-CH3 region as described for the derivation of pDB952.

PCR products of approximately 700bp (hinge-CH2-CH3 of IgG4 PE mutant) and 400bp (IL4.Y124D) are obtained and purified using the Promega "Magic PCR cleanup" kit. The purified PCR reactions are then digested with the following enzymes to create "sticky ends": XhoI and XbaI for IgG4 PE and EcoRV and XhoI for IL4.Y124D. The digests are incubated at 37°C for 3 hours and then ethanol precipitated. The resulting DNAs are of sizes of approximately 690bp (hinge-CH2-CH3 of IgG4 PE) and 370bp (IL4.Y124D).

To obtain larger amounts of the IgG4 PE variant hinge-CH2-CH3 fragment and the IL4.Y124D fragment, the purified and digested PCR products are ligated into Bluescript KS+™ which is prepared by digestion with either XhoI and XbaI for the hinge-CH2-CH3 of IgG4 PE fragment or EcoRV and XhoI for the IL4.Y124D fragment, followed by Geneclean™. A Bluescript KS+/hinge-CH2-CH3 of IgG4 PE recombinant and a Bluescript KS+/IL4.Y124D recombinant are thus generated. Large amounts of these DNAs are produced using the Promega "Magic Maxiprep" method. The IgG4 PE hinge-CH2-CH3 fragment is excised from the Bluescript recombinant using XhoI and XbaI. The resulting fragment of approximately 690bp is purified by Geneclean™ to generate large amounts of the IgG4 PE hinge-CH2-CH3 XhoI/XbaI fragment. The IL4.Y124D fragment is excised from the Bluescript recombinant using EcoRV and XhoI and the resulting fragment of approximately 370bp is purified by Geneclean™.

A vector is prepared into which to ligate the hinge-CH2-CH3 of IgG4 PE and IL4.Y124D fragments by digesting pDB951 with EcoRV and XbaI as described for the derivation of pDB952.

The three fragments described (IL4.Y124D EcoRV/XhoI, hinge-CH2-CH3 of IgG4 PE variant XhoI/XbaI and the 5.8Kb fragment resulting from EcoRV/XbaI digestion of pDB951) are ligated together to form plasmid pDB953 using a DNA ligation kit from Amersham (product code RPN 1507), incubating the

reactions at 16°C overnight. The ligation reaction products are transformed into Promega JM109 competent cells (high efficiency) and plated onto Luria Broth agar containing ampicillin at 50μg/ml. Transformants are cultured in Luria Broth (containing ampicillin at 50μg/ml) and DNA prepared using Promega "Magic Minipreps". Production of an IL4.Y124D/IgG4 PE variant recombinant DNA is verified by restriction digests, and the complete IL4.Y124D and hinge-CH2-CH3 IgG4 PE variant regions are verified by DNA sequencing. Table 9 describes the sequence of the coding region only of the IL4.Y124D/IgG4 PE fusion molecule, and Table 10 contains the amino acid sequence of the fusion protein. The IL4.Y124D/IgG4 PE recombinant DNA is prepared and purified using caesium chloride gradients and the DNA used to transiently transfect HeLa cells.

2. Expression of the fusion protein

HeLa cells were grown in MEMα medium (Gibco) with 10% foetal calf serum and 1% glutamine. For the assay, 1 x 10^6 HeLa cells were seeded in 15mls RPMI-1640 medium with 10% newborn calf serum, 1% glutamine ("seeding medium"), in a 75cm^2 flask, four days prior to transfection. On the day prior to transfection, a further 12.5mls seeding medium was added to each flask. On the day of transfection, the medium was changed to 15mls of "transfection medium" (MEM medium with Earle's salts containing 10% newborn calf serum and 1% non essential amino acids), at time zero. At time +3 hours, 25μg of the appropriate DNA in 0.125M $CaCl_2$, 1x HBS (HEPES buffered saline) was added to the cells. At time +7 hours, the cells were subjected to a glycerol shock (15%v/v) and then left to incubate overnight in 12.5mls seeding medium containing 5mM sodium butyrate. The next day the cells were washed with PBS (Dulbecco's phosphate buffered saline) and 12.5mls "harvest medium" (RPMI-1640 with 2% of a 7.5% stock sodium bicarbonate solution) was added. After a further 24 hour incubation, the supernatants were removed, centrifuged at 1000rpm for 5 minutes to remove cell debris and stored at either 4°C or -20°C.

3. Biological Activity

For assay of supernatant for IL4 antagonist activity: using the method described in Spits et al., J. Immunology <u>139</u>, 1142 (1987), human peripheral blood lymphocytes were incubated for three days with phytohaemagluttinin, a T cell mitogen, to upregulate the IL4 receptor. The resultant blast cells were then stimulated for a further three days with IL4. Proliferation was measured by the incorporation of 3H thymidine.

The IL4.Y124D/IgG4 PE chimera inhibited ^3H thymidine incorporation by human peripheral blood T lymphocytes stimulated with 133pM IL4 in a dose dependent manner.

Example 4. Mammalian Expression vector containing DNA coding for IL4.Y124D/IgG4 PE.

1. Construction of DNA

The pCDN vector (Aiyar, N., Baker, E., Wu, H-L., Nambi, P., Edwards, R.M., Trill, J.J., Ellis, C., Bergsma, D. Molecular and Cellular Biochemistry 131:75-86, 1994) contains the CMV promoter, a polylinker cloning region, and the BGH polyadenylation region. This vector also contains a bacterial neomycin phosphotransferase gene (NEO) inserted between the β-globin promoter and SV40 polyadenylation region for Geneticin™ selection, the DHFR selection cassette inserted between the β-globin promoter and BGH polydenylation region for methotrexate (MTX) amplification, an ampicillin resistance gene for growth in bacteria, and a SV40 origin of replication.

To insert the IL4.Y124D/IgG4 PE cDNA, the pCDN vector was prepared by digesting with Nde1 and BstX1 as follows: 15μg of DNA was incubated with 30 units of BstX1 in react 2 (Gibco-BRL) at 55°C for 1 hour, and ethanol precipitated. The resulting DNA was digested with Nde1 in react 2 at 37°C for 1 hour, and ethanol precipitated. The IL4.Y124D/IgG4 PE fragment was prepared from pDB953 (Example 3.1) by digesting with BstX1 and Nde1 as follows: 15μg of DNA was incubated with 30 units of BstX1 in react 2 at 55°C for 1 hour, and ethanol precipitated. The resulting DNA was digested with Nde1 in react 2 at 37°C for 1 hour, and ethanol precipitated.

The IL4.Y124D/IgG4 PE Nde1/BstX1 and pCDN Nde1/BstX1 fragments were ligated together to form the plasmid pCDN-IL4.Y124D/IgG4 PE. The ligation was achieved using 2 units of T4 DNA Ligase (Gibco BRL) with T4 DNA Ligase buffer. The reactions were incubated at 16°C overnight. The ligation reaction products were transformed into Gibco-BRL DH5a competent cells (subcloning efficiency) and plated onto Luria Broth agar containing 75 ug/ml ampicillin. Transformants were cultured in Luria Broth (containing ampicillin at 50 ug/ml) and DNA prepared by alkaline lysis. Production of a pCDN-IL4.Y124D/IgG4 PE DNA was confirmed by restriction digests. The complete sequence of the recombinant IL4.Y124D/IgG4 PE DNA was confirmed by sequencing. The pCDN-IL4.Y124D/IgG4 PE recombinant DNA was prepared and purified using Qiagen columns and the DNA was used to transiently infect COS cells and electroporated into CHO cells to create stable clones.

2. Expression of the Fusion Protein

a) Transient Expression in COS

COS-1 cells were grown in DMEM medium with 10% fetal bovine serum. For the transfection, cells were seeded at 2 X 10^5 cells into a 35mm tissue culture dish 24 hours prior. A solution containing 1μg of DNA in100ul of DMEM without serum is added to a solution containing 6μl of LIPOFECTAMINE Reagent (Gibco-BRL) in 100μl of DMEM without serum, gently swirled and incubated at room temperature for 45 minutes. The cells are washed once with serum free DMEM. 0.8ml of serum free DMEM is added to the DNA-LIPOFECTAMINE SOLUTION, mixed gently and the diluted solution is overlayed on the cells. The cells are incubated at 37°C for 5 hours, then 1ml of DMEM containing 20% fetal bovine serum is added. The cells are assayed 48-72 hours later to determine expression levels.

b) Electroporation into CHO cells

CHO cells, ACC-098 (a suspension cell line derived from CHO DG-44, Urlaub, G., Kas, E., Carothers, A.M. and Chasin, L.A. Cell, 33. 405-412, 1983) were grown in serum free growth medium WO 92/05246. 15μg of the pCDN-IL4.Y124D/IgG4 PE plasmid was digested using 30 units of Not1 at 37°C for 3 hours to linearize the plasmid, and precipitated with ethanol. The resulting DNA was resuspended in 50ul of 1 X TE (10mM Tris, pH 8.0, 1mM EDTA). The DNA was electroporated into 1 X 10^7 ACC-098 cells, using a Bio Rad Gene Pulser set at 380V and 25μFd. The cells were resupended into growth medium at 2.5 X 10^4 cells/ml, and 200μl of the cell suspension was plated into each well of a 96 well plate. 48 hours later the medium was switched to growth medium containing 400μ g/ml G418 (Geneticin). Twenty one days post selection, conditioned medium from the colonies which arose were screened by Elisa assay. The highest expressing colonies were transferred to 24 well plates in order to be scaled up.

Table 1. DNA sequence of COSFcLink vector, 6367bp

SEQ ID No:1

```
GACGTCGACGGATCGGGAGATCGGGGATCGATCCGTCGACGTACGACTAGTTATTAATAG   60
TAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTT  120
ACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATG  180
ACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTAT  240
TTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCT  300
ATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGG  360
GACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGG  420
TTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTC  480
CACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAA  540
TGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTC  600
TATATAAGCAGAGCTGGGTACGTGAACCGTCAGATCGCCTGGAGACGCCATCGAATTCGG  660
TTACCTGCAGATATCAAGCTAATTCGGTACCGAGCCCAAATCGGCCGACAAAACTCACAC  720
ATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC  780
AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGA  840
CGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCA  900
TAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGT  960
CCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAA 1020
CAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGA 1080
ACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCT 1140
GACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGG 1200
GCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTT 1260
CCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG 1320
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCC 1380
GGGTAAATGAGTGTAGTCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCA 1440
GCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCAC 1500
TGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTAT 1560
TCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCA 1620
TGCTGGGGATGCGGTGGGCTCTATGGAACCAGCTGGGGCTCGAGGGGGGATCTCCCGATC 1680
CCCAGCTTTGCTTCTCAATTTCTTATTTGCATAATGAGAAAAAAAGGAAAATTAATTTTA 1740
ACACCAATTCAGTAGTTGATTGAGCAAATGCGTTGCCAAAAAGGATGCTTTAGAGACAGT 1800
GTTCTCTGCACAGATAAGGACAAACATTATTCAGAGGGAGTACCCAGAGCTGAGACTCCT 1860
AAGCCAGTGAGTGGCACAGCATTCTAGGGAGAAATATGCTTGTCATCACCGAAGCCTGAT 1920
TCCGTAGAGCCACACCTTGGTAAGGGCCAATCTGCTCACACAGGATAGAGAGGGCAGGAG 1980
CCAGGGCAGAGCATATAAGGTGAGGTAGGATCAGTTGCTCCTCACATTTGCTTCTGACAT 2040
AGTTGTGTTGGGAGCTTGGATAGCTTGGACAGCTCAGGGCTGCGATTTCGCGCCAAACTT 2100
GACGGCAATCCTAGCGTGAAGGCTGGTAGGATTTTATCCCCGCTGCCATCATGGTTCGAC 2160
CATTGAACTGCATCGTCGCCGTGTCCCAAAATATGGGGATTGGCAAGAACGGAGACCTAC 2220
CCTGGCCTCCGCTCAGGAACGAGTTCAAGTACTTCCAAAGAATGACCACAACCTCTTCAG 2280
TGGAAGGTAAACAGAATCTGGTGATTATGGGTAGGAAAACCTGGTTCTCCATTCCTGAGA 2340
AGAATCGACCTTTAAAGGACAGAATTAATATAGTTCTCAGTAGAGAACTCAAAGAACCAC 2400
CACGAGGAGCTCATTTTCTTGCCAAAAGTTTGGATGATGCCTTAAGACTTATTGAACAAC 2460
CGGAATTGGCAAGTAAAGTAGACATGGTTTGGATAGTCGGAGGCAGTTCTGTTTACCAGG 2520
AAGCCATGAATCAACCAGGCCACCTTAGACTCTTTGTGACAAGGATCATGCAGGAATTTG 2580
AAAGTGACACGTTTTTCCCAGAAATTGATTTGGGGAAATATAAACTTCTCCCAGAATACC 2640
CAGGCGTCCTCTCTGAGGTCCAGGAGGAAAAAGGCATCAAGTATAAGTTTGAAGTCTACG 2700
AGAAGAAAGACTAACAGGAAGATGCTTTCAAGTTCTCTGCTCCCCTCCTAAAGCTATGCA 2760
TTTTTATAAGACCATGCTAGCTTGAACTTGTTTATTGCAGCTTATAATGGTTACAAATAA 2820
AGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGT 2880
TTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCAACGATAGCTTATCTGTGGGC 2940
GATGCCAAGCACCTGGATGCTGTTGGTTTCCTGCTACTGATTTAGAAGCCATTTGCCCCC 3000
```

TGAGTGGGGCTTGGGAGCACTAACTTTCTCTTTCAAAGGAAGCAATGCAGAAAGAAAAGC 3060
ATACAAAGTATAAGCTGCCATGTAATAATGGAAGAAGATAAGGTTGTATGAATTAGATTT 3120
ACATACTTCTGAATTGAAACTAAACACCTTTAAATTCTTAAATATATAACACATTTCATA 3180
TGAAAGTATTTTACATAAGTAACTCAGATACATAGAAAACAAAGCTAATGATAGGTGTCC 3240
CTAAAAGTTCATTTATTAATTCTACAAATGATGAGCTGGCCATCAAAATTCCAGCTCAAT 3300
TCTTCAACGAATTAGAAAGAGCAATCTGCAAACTCATCTGGAATAACAAAAAACCTAGGA 3360
TAGCAAAAACTCTTCTCAAGGATAAAAGAACCTCTGGTGGAATCACCATGCCTGACCTAA 3420
AGCTGTACTACAGAGCAATTGTGATAAAAACTGCATGGTACTGATATAGAAACGGACAAG 3480
TAGACCAATGGAATAGAACCCACACACCTATGGTCACTTGATCTTCAACAAGAGAGCTAA 3540
AACCATCCACTGGAAAAAAGACAGCATTTTCAACAAATGGTGCTGGCACAACTGGTGGTT 3600
ATCATGGAGAAGAATGTGAATTGATCCATTCCAATCTCCTTGTACTAAGGTCAAATCTAA 3660
GTGGATCAAGGAACTCCACATAAAACCAGAGACACTGAAACTTATAGAGGAGAAAGTGGG 3720
GAAAAGCCTCGAAGATATGGGCACAGGGGAAAAATTCCTGAATAGAACAGCAATGGCTTG 3780
TGCTGTAAGATCGAGAATTGACAAATGGGACCTCATGAAACTCCAAAGCTATCGGATCAA 3840
TTCCTCCAAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCGAGGCGGCCTCGG 3900
CCTCTGCATAAATAAAAAAAATTAGTCAGCCATGCATGGGGCGGAGAATGGGCGGAACTG 3960
GGCGGAGTTAGGGGCGGGATGGGCGGAGTTAGGGGCGGGACTATGGTTGCTGACTAATTG 4020
AGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCTGGTT 4080
GCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTT 4140
CCACACCCTAACTGACACACATTCCACAGAATTAATTCCCGATCCCGTCGACCTCGAGAG 4200
CTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCC 4260
ACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTA 4320
ACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCA 4380
GCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTC 4440
CGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGC 4500
TCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACAT 4560
GTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTT 4620
CCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCG 4680
AAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTC 4740
TCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGT 4800
GGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAA 4860
GCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTA 4920
TCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAA 4980
CAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAA 5040
CTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTT 5100
CGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTT 5160
TTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGAT 5220
CTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCAT 5280
GAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATC 5340
AATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGC 5400
ACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTA 5460
GATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGA 5520
CCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCG 5580
CAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGC 5640
TAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCAT 5700
CGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAG 5760
GCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGAT 5820
CGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAA 5880
TTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAA 5940
GTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGA 6000
TAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGG 6060
GCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGC 6120
ACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGG 6180
AAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACT 6240

CTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACAT 6300
ATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGT 6360
GCCACCT 6367

Table 2. DNA sequence of encoded Y124D-IgG1 fusion molecule in COSFcLink vector, 6926bp

SEQ ID No:2

GACGTCGACGGATCGGGAGATCGGGGATCGATCCGTCGACGTACGACTAGTTATTAATAG 60
TAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTT 120
ACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATG 180
ACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTAT 240
TTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCT 300
ATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGG 360
GACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGG 420
TTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTC 480
CACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAA 540
TGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTC 600
TATATAAGCAGAGCTGGGTACGTGAACCGTCAGATCGCCTGGAGACGCCATCGAATTCGG 660
TTACCTGCAGATGGGCTGCAGGAATTCCGCATTGCAGAGATAATTGTATTTAAGTGCCTA 720
GCTCGATACAATAAACGCCATTTGACCATTCACCACATTGGTGTGCACCTCCAAGCTTAC 780
CTGCCATGGGTCTCACCTCCCAACTGCTTCCCCCTCTGTTCTTCCTGCTAGCATGTGCCG 840
GCAACTTTGTCCACGGACACAAGTGCGATATCACCTTACAGGAGATCATCAAAACTTTGA 900
ACAGCCTCACAGAGCAGAAGACTCTGTGCACCGAGTTGACCGTAACAGACATCTTTGCTG 960
CCTCCAAGAACACAACTGAGAAGGAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGCAGT 1020
TCTACAGCCACCATGAGAAGGACACTCGCTGCCTGGGTGCGACTGCACAGCAGTTCCACA 1080
GGCACAAGCAGCTGATCCGATTCCTGAAACGGCTCGACAGGAACCTCTGGGGCCTGGCGG 1140
GCTTGAATTCCTGTCCTGTGAAGGAAGCCAACCAGAGTACGTTGGAAAACTTCTTGGAAA 1200
GGCTAAAGACGATCATGAGAGAGAAAGACTCAAAGTGTTCGAGCGGTACCGAGCCCAAAT 1260
CGGCCGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGT 1320
CAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGG 1380
TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACG 1440
TGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA 1500
CGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGT 1560
ACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAG 1620
CCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGA 1680
CCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCG 1740
TGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGG 1800
ACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGC 1860
AGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGA 1920
AGAGCCTCTCCCTGTCTCCGGGTAAATGAGTGTAGTCTAGAGCTCGCTGATCAGCCTCGA 1980
CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCC 2040
TGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTC 2100
TGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATT 2160
GGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAACCAGCTGGGGCTC 2220
GAGGGGGGATCTCCCGATCCCCAGCTTTGCTTCTCAATTTCTTATTTGCATAATGAGAAA 2280
AAAAGGAAAATTAATTTTAACACCAATTCAGTAGTTGATTGAGCAAATGCGTTGCCAAAA 2340
AGGATGCTTTAGAGACAGTGTTCTCTGCACAGATAAGGACAAACATTATTCAGAGGGAGT 2400
ACCCAGAGCTGAGACTCCTAAGCCAGTGAGTGGCACAGCATTCTAGGGAGAAATATGCTT 2460
GTCATCACCGAAGCCTGATTCCGTAGAGCCACACCTTGGTAAGGGCCAATCTGCTCACAC 2520

AGGATAGAGAGGGCAGGAGCCAGGGCAGAGCATATAAGGTGAGGTAGGATCAGTTGCTCC 2580
TCACATTTGCTTCTGACATAGTTGTGTTGGGAGCTTGGATAGCTTGGACAGCTCAGGGCT 2640
GCGATTTCGCGCCAAACTTGACGGCAATCCTAGCGTGAAGGCTGGTAGGATTTTATCCCC 2700
GCTGCCATCATGGTTCGACCATTGAACTGCATCGTCGCCGTGTCCCAAAATATGGGGATT 2760
GGCAAGAACGGAGACCTACCCTGGCCTCCGCTCAGGAACGAGTTCAAGTACTTCCAAAGA 2820
ATGACCACAACCTCTTCAGTGGAAGGTAAACAGAATCTGGTGATTATGGGTAGGAAAACC 2880
TGGTTCTCCATTCCTGAGAAGAATCGACCTTTAAAGGACAGAATTAATATAGTTCTCAGT 2940
AGAGAACTCAAAGAACCACCACGAGGAGCTCATTTTCTTGCCAAAAGTTTGGATGATGCC 3000
TTAAGACTTATTGAACAACCGGAATTGGCAAGTAAAGTAGACATGGTTTGGATAGTCGGA 3060
GGCAGTTCTGTTTACCAGGAAGCCATGAATCAACCAGGCCACCTTAGACTCTTTGTGACA 3120
AGGATCATGCAGGAATTTGAAAGTGACACGTTTTTCCCAGAAATTGATTTGGGGAAATAT 3180
AAACTTCTCCCAGAATACCCAGGCGTCCTCTCTGAGGTCCAGGAGGAAAAAGGCATCAAG 3240
TATAAGTTTGAAGTCTACGAGAAGAAAGACTAACAGGAAGATGCTTTCAAGTTCTCTGCT 3300
CCCCTCCTAAAGCTATGCATTTTTATAAGACCATGCTAGCTTGAACTTGTTTATTGCAGC 3360
TTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTC 3420
ACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCAA 3480
CGATAGCTTATCTGTGGGCGATGCCAAGCACCTGGATGCTGTTGGTTTCCTGCTACTGAT 3540
TTAGAAGCCATTTGCCCCCTGAGTGGGGCTTGGGAGCACTAACTTTCTCTTTCAAAGGAA 3600
GCAATGCAGAAAGAAAAGCATACAAAGTATAAGCTGCCATGTAATAATGGAAGAAGATAA 3660
GGTTGTATGAATTAGATTTACATACTTCTGAATTGAAACTAAACACCTTTAAATTCTTAA 3720
ATATATAACACATTTCATATGAAAGTATTTTACATAAGTAACTCAGATACATAGAAAACA 3780
AAGCTAATGATAGGTGTCCCTAAAAGTTCATTTATTAATTCTACAAATGATGAGCTGGCC 3840
ATCAAAATTCCAGCTCAATTCTTCAACGAATTAGAAAGAGCAATCTGCAAACTCATCTGG 3900
AATAACAAAAAACCTAGGATAGCAAAAACTCTTCTCAAGGATAAAAGAACCTCTGGTGGA 3960
ATCACCATGCCTGACCTAAAGCTGTACTACAGAGCAATTGTGATAAAAACTGCATGGTAC 4020
TGATATAGAAACGGACAAGTAGACCAATGGAATAGAACCCACACACCTATGGTCACTTGA 4080
TCTTCAACAAGAGAGCTAAAACCATCCACTGGAAAAAAGACAGCATTTTCAACAAATGGT 4140
GCTGGCACAACTGGTGGTTATCATGGAGAAGAATGTGAATTGATCCATTCCAATCTCCTT 4200
GTACTAAGGTCAAATCTAAGTGGATCAAGGAACTCCACATAAAACCAGAGACACTGAAAC 4260
TTATAGAGGAGAAAGTGGGGAAAAGCCTCGAAGATATGGCACAGGGGAAAAATTCCTGA 4320
ATAGAACAGCAATGGCTTGTGCTGTAAGATCGAGAATTGACAAATGGACCTCATGAAAC 4380
TCCAAAGCTATCGGATCAATTCCTCCAAAAAAGCCTCCTCACTACTTCTGGAATAGCTCA 4440
GAGGCCGAGGCGGCCTCGGCCTCTGCATAAATAAAAAAAATTAGTCAGCCATGCATGGGG 4500
CGGAGAATGGGCGGAACTGGGCGGAGTTAGGGGCGGGATGGGCGGAGTTAGGGGCGGGAC 4560
TATGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGG 4620
GGACTTTCCACACCTGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGC 4680
TGGGGAGCCTGGGGACTTTCCACACCCTAACTGACACACATTCCACAGAATTAATTCCCG 4740
ATCCCGTCGACCTCGAGAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATT 4800
GTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGG 4860
GTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGT 4920
CGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTT 4980
TGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGC 5040
TGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGG 5100
ATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGG 5160
CCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGAC 5220
GCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTG 5280
GAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCT 5340
TTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGG 5400
TGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCT 5460
GCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCAC 5520
TGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGT 5580
TCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTC 5640
TGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCA 5700
CCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGAT 5760

```
CTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCAC   5820
GTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATT   5880
AAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACC   5940
AATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTG   6000
CCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTG   6060
CTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGC   6120
CAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTA   6180
TTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTG   6240
TTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCT   6300
CCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTA   6360
GCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGG   6420
TTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGA   6480
CTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTT   6540
GCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCA   6600
TTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTT   6660
CGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTT   6720
CTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGA   6780
AATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATT   6840
GTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGC   6900
GCACATTTCCCCGAAAAGTGCCACCT                                     6926
```

Table 3. DNA sequence of IL4.Y124D/IgG1 fusion molecule coding region, 1164bp

SEQ ID No:3

```
ATGGGTCTCACCTCCCAACTGCTTCCCCCTCTGTTCTTCCTGCTAGCATGTGCCGGCAAC     60
TTTGTCCACGGACACAAGTGCGATATCACCTTACAGGAGATCATCAAAACTTTGAACAGC    120
CTCACAGAGCAGAAGACTCTGTGCACCGAGTTGACCGTAACAGACATCTTTGCTGCCTCC    180
AAGAACACAACTGAGAAGGAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGCAGTTCTAC    240
AGCCACCATGAGAAGGACACTCGCTGCCTGGGTGCGACTGCACAGCAGTTCCACAGGCAC    300
AAGCAGCTGATCCGATTCCTGAAACGGCTCGACAGGAACCTCTGGGGCCTGGCGGGCTTG    360
AATTCCTGTCCTGTGAAGGAAGCCAACCAGAGTACGTTGGAAAACTTCTTGGAAAGGCTA    420
AAGACGATCATGAGAGAGAAAGACTCAAAGTGTTCGAGCGGTACCGAGCCCAAATCGGCC    480
GACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTC    540
TTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACA    600
TGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGAC    660
GGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTAC    720
CGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAG    780
TGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAA    840
GGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAG    900
AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAG    960
TGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCC   1020
GACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGG   1080
AACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGC   1140
CTCTCCCTGTCTCCGGGTAAATGA                                       1164
```

Table 4. Sequence of encoded IL4.Y124D/IgG1 fusion protein, 387aa

SEQ ID No:4

```
 1  MGLTSQLLPP LFFLLACAGN FVHGHKCDIT LQEIIKTLNS LTEQKTLCTE
51  LTVTDIFAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH
```

```
101  KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKDSK
151  CSSGTEPKSA DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT
201  CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH
251  QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
301  NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL
351  TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK*
```

Table 5. DNA sequence of synthetic IgG4 cDNA, 1006bp

SEQ ID No:5

```
GCTTCCACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAG      60
AGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCG     120
TGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA     180
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGACC     240
TACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCC     300
AAATATGGTCCCCCATGCCCATCATGCCCAGCACCTGAATTTCTGGGGGGACCATCAGTC     360
TTCCTGTTCCCCCCAAAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACG     420
TGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGAT     480
GGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC     540
CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAG     600
TGCAAGGTCTCCAACAAAGGCCTCCCGTCATCGATCGAGAAAACCATCTCCAAAGCCAAA     660
GGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAG     720
AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAG     780
TGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCC     840
GACGGATCCTTCTTCCTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGG     900
AATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGC     960
CTCTCCCTGTCTCTGGGTAAATGAGTGTAGTCTAGATCTACGTATG                  1006
```

Table 6. DNA sequence of IL4.Y124D/IgG4 fusion molecule coding region, 1149bp

SEQ ID No:6

```
ATGGGTCTCACCTCCCAACTGCTTCCCCCTCTGTTCTTCCTGCTAGCATGTGCCGGCAAC      60
TTTGTCCACGGACACAAGTGCGATATCACCTTACAGGAGATCATCAAAACTTTGAACAGC     120
CTCACAGAGCAGAAGACTCTGTGCACCGAGTTGACCGTAACAGACATCTTTGCTGCCTCC     180
AAGAACACAACTGAGAAGGAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGCAGTTCTAC     240
AGCCACCATGAGAAGGACACTCGCTGCCTGGGTGCGACTGCACAGCAGTTCCACAGGCAC     300
AAGCAGCTGATCCGATTCCTGAAACGGCTCGACAGGAACCTCTGGGGCCTGGCGGGCTTG     360
AATTCCTGTCCTGTGAAGGAAGCCAACCAGAGTACGTTGGAAAACTTCTTGGAAAGGCTA     420
AAGACGATCATGAGAGAGAAAGACTCAAAGTGCTCGAGCGAGTCCAAATATGGTCCCCCA     480
TGCCCATCATGCCCAGCACCTGAATTTCTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA     540
AAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGAC     600
GTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCAT     660
AATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTC     720
CTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAAC     780
AAAGGCCTCCCGTCATCGATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAG     840
CCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTG     900
ACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG     960
CAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGATCCTTCTTC    1020
CTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGC    1080
TCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTG    1140
```

```
GGTAAATGA                                                     1149
```

Table 7. Sequence of encoded IL4.Y124D/IgG4 fusion protein, 382aa

SEQ ID No:7

```
  1  MGLTSQLLPP LFFLLACAGN FVHGHKCDIT LQEIIKTLNS LTEQKTLCTE
 51  LTVTDIFAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH
101  KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKDSK
151  CSSESKYGPP CPSCPAPEFL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
201  VSQEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTYRVVSV LTVLHQDWLN
251  GKEYKCKVSN KGLPSSIEKT ISKAKGQPRE PQVYTLPPSQ EEMTKNQVSL
301  TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSRLTVDKS
351  RWQEGNVFSC SVMHEALHNH YTQKSLSLSL GK*
```

Table 8. DNA sequence of IgG4 PE variant, 984bp

SEQ ID No:8

```
GCTAGTACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAG    60
AGCACgGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCG   120
TGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA   180
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGACC   240
TACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCC   300
AAATATGGTCCCCCATGCCCAcCATGCCCAGCgCCTGAaTTtgaGGGGGGACCATCAGTC   360
TTCCTGTTCCCCCCAAAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACG   420
TGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGAT   480
GGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC   540
CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAG   600
TGCAAGGTCTCCAACAAAGGCCTCCCGTCaTCgATCGAGAAAACCATCTCCAAAGCCAAA   660
GGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAG   720
AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAG   780
TGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCC   840
GACGGaTCCTTCTTCCTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGG   900
AATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGC   960
CTCTCCCTGTCTCTGGGTAAATGA                                       984
```

Table 9. DNA sequence of IL4.Y124D/IgG4 PE fusion molecule coding region, 1149bp

SEQ ID No:9

```
ATGGGTCTCACCTCCCAACTGCTTCCCCCTCTGTTCTTCCTGCTAGCATGTGCCGGCAAC    60
TTTGTCCACGGACACAAGTGCGATATCACCTTACAGGAGATCATCAAAACTTTGAACAGC   120
CTCACAGAGCAGAAGACTCTGTGCACCGAGTTGACCGTAACAGACATCTTTGCTGCCTCC   180
AAGAACACAACTGAGAAGGAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGCAGTTCTAC   240
AGCCACCATGAGAAGGACACTCGCTGCCTGGGTGCGACTGCACAGCAGTTCCACAGGCAC   300
AAGCAGCTGATCCGATTCCTGAAACGGCTCGACAGGAACCTCTGGGGCCTGGCGGGCTTG   360
AATTCCTGTCCTGTGAAGGAAGCCAACCAGAGTACGTTGGAAAACTTCTTGGAAAGGCTA   420
AAGACGATCATGAGAGAGAAAGACTCAAAGTGCTCGAGCGAGTCCAAATATGGTCCCCCA   480
TGCCCACCATGCCCAGCgCCTGAATTTGAGGGGGGACCATCAGTCTTCCTGTTCCCCCCA   540
```

```
AAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGAC  600
GTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCAT  660
AATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTC  720
CTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAAC  780
AAAGGCCTCCCGTCaTCgATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAG  840
CCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTG  900
ACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG  960
CAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGaTCCTTCTTC 1020
CTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGC 1080
TCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTG 1140
GGTAAATGA                                                    1149
```

Table 10. Sequence of encoded IL4.Y124D/IgG4 PE variant fusion protein, 382aa

SEQ ID No:10

```
  1 MGLTSQLLPP LFFLLACAGN FVHGHKCDIT LQEIIKTLNS LTEQKTLCTE
 51 LTVTDIFAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH
101 KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKDSK
151 CSSESKYGPP CPPCPAPEFE GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
201 VSQEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTYRVVSV LTVLHQDWLN
251 GKEYKCKVSN KGLPSSIEKT ISKAKGQPRE PQVYTLPPSQ EEMTKNQVSL
301 TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSRLTVDKS
351 RWQEGNVFSC SVMHEALHNH YTQKSLSLSL GK*
```